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The ultrastructure of the central nucleus of the inferior colliculus of the genetically epilepsy-prone rat

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The inferior colliculus of the genetically epilepsy-prone rat (GEPR) was examined at the ultrastructural level to determine if any abnormalities exist in the inferior colliculus of the GEPR as compared to the non-epileptic Sprague–Dawley rat. Both routine electron microscopic preparations and glutamate decarboxylase (GAD) and GABA immunocytochemical preparations were examined in the GEPR and compared to previous studies from this laboratory that described the normal ultrastructure of the Sprague–Dawley rat. Cell counts from 2 μm semi-thin sections confirmed our previous observations that showed a large, significant increase in the number of neurons in the inferior colliculus of the GEPR as compared to the Sprague–Dawley rat. Many of the small neurons in the inferior colliculus of the GEPR were found to be smaller than those in the inferior colliculus of the Sprague–Dawley rat. Moreover, the small neurons in the GEPR were frequently clumped in clusters of 3–5.

Several ultrastructural abnormalities present in the inferior colliculus of the GEPR have been observed at epileptic foci or in brain regions along the pathway of seizure spread in other experimental models of epilepsy. These changes included the presence of dendrites which are almost completely devoid of organelles, hypertrophy of glial processes, and terminals that contain either swollen vesicles or very few vesicles. Other features that were frequently observed in the GEPR but were rarely found in preparations of Sprague–Dawley rats included an abundance of extra membranes, whorl bodies and multivesicular bodies within somata, dendrites and axons. Symmetric and asymmetric axosomatic synapses were counted in the inferior colliculus of both the GEPR and Sprague–Dawley rats, and the results indicated that the number of these synapses for each of the various cell types was not significantly different between the Sprague–Dawley rat and the GEPR. Many of the abnormally small neurons in the GEPR were shown to be GABAergic in the immunocytochemical preparations. Apart from this aspect, the morphology of the GABAergic neurons and axon terminals in the inferior colliculus of the GEPR appeared similar to those in the inferior colliculus of the Sprague–Dawley rat.

INTRODUCTION

The inferior colliculus of the genetically epilepsy-prone rat (GEPR) is required for the expression of audiogenic seizures[10,38] and it exhibits several abnormalities. For example, γ-aminobutyric acid (GABA) and benzodiazepines are less effective in the inferior colliculus of the GEPR than in the inferior colliculus of the non-epileptic Sprague–Dawley rat[2,3]. Neurons in the inferior colliculus exhibit elevated thresholds to sound and a smaller degree of firing reduction during binaural inhibition than do units in the normal Sprague–Dawley

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rat[4]. In addition, neurons in the inferior colliculus of the GEPR show an increase in afterdischarge-like responses similar to those seen in other types of epilepsies[4]. In fact, afterdischarges are recorded from the inferior colliculus, but not from the cortex, during wild running behavior[12]. The results of a recent biochemical study have shown a significant increase in the level of GABA in the inferior colliculus of the GEPR[25]. Consistent with this finding is the observation of an increase in the number of GABAergic neurons as well as an increase in the total number of neurons in the inferior colliculus of the GEPR as compared to the Sprague–Dawley rat[34]. This anatomical feature is not caused by the seizures because the increase in cell number is present prior to the time that seizure activity commences[31]. This increase in cell number is inherited with seizure behavior because it is present in only the seizing offspring of GEPR × Sprague–Dawley crosses[28]. Thus, this anatomical feature may be a determinant of seizure activity.

Another genetic model of epilepsy, the seizure-sensitive gerbil, also exhibits an increase in the number of GABAergic neurons, but it occurs in the dentate gyrus of the hippocampus[21]. This increase is also found in young offspring of seizure-sensitive gerbils at an age prior to the onset of seizure behavior[21]. Seizure-sensitive gerbils exhibit generalized clonic convulsions in response to being placed in a novel environment, a procedure which is most likely stressful to the animal[11]. In this model, an increase in the number of GABAergic basket cells occurs in the dentate gyrus of the hippocampus, and the integrity of the perforant path, but not of the fornix, is necessary for the manifestation of seizures[26]. Therefore, certain similarities exist between these 2 genetic models of epilepsy.

Peterson et al.[21] suggested a hypothesis of disinhibition to explain how an increase in GABAergic neurons might be responsible for seizure behavior. Briefly, these investigators reasoned that if the increase in GABAergic neurons resulted in the increased inhibition of other GABAergic cells, which normally provide tonic inhibition to excitatory projection neurons, then the result would be a disinhibition of projection neurons. Preliminary data support this hypothesis in that the hippocampus of the seizure-sensitive gerbil displays an increase in the number of GABAergic, symmetric axosomatic synapses on the somata of basket cells[20]. Although the abnormalities in the central nervous system of the GEPR are complex, it is possible that such a circuitry may occur in the inferior colliculus of the GEPR and could be a structural basis for seizure behavior emanating from abnormal activity in the inferior colliculus.

The present study was undertaken to investigate the disinhibition hypothesis in the GEPR and to determine whether other ultrastructural changes are present in the inferior colliculus of the GEPR as compared to the inferior colliculus of the non-epileptic Sprague–Dawley rat. Thus, both routine electron microscopic preparations and GABA and glutamic acid decarboxylase (GAD) immunocytochemical preparations were examined. Axosomatic and axodendritic synapses were counted for the various cell types present in the GEPR preparations and for similar cells in Sprague–Dawley preparations[27]. In addition, the preparations were examined for ultrastructural abnormalities because morphological changes have been reported at epileptic foci in some experimental models of epilepsy[14,17–19,21–24]. A preliminary report of these findings was presented at the 16th annual meeting of the Society for Neuroscience[32].

METHODS

Animals

Sixteen adult GEPRs born in our colony and 4 Sprague–Dawley rats were used in this study. All rats were between 3 and 6 months of age at the time of sacrifice. Both Sprague–Dawley rats and GEPRs were subjected to at least 3 sound stimulation tests as previously described[34], and the seizure behavior was rated according to the scale of Jobe et al.[9]. All GEPRs used in this study consistently exhibited maximal tonic-clonic seizures. In contrast, the Sprague–Dawley rats never displayed seizures.

Fixatives

The animals were sacrificed 1 week after their last sound stimulation test. Eight GEPRs and 4 Sprague–Dawley rats were perfused for normal ultrastructural analysis according to the method of

Friedrich and Mugnaini[6] that utilizes a 3-stage perfusion protocol (fixative no. 1): (1) 100 ml/rat of Ringer's solution containing 0.85% NaCl, 0.25% KCl and 0.02% $NaHCO_3$ (pH 7.3, 37 °C); (2) 500 ml/rat of 1% paraformaldehyde and 1.25% glutaraldehyde in 0.12 M phosphate buffer (pH 7.3, 25 °C); (3) 500 ml/rat of 3% glutaraldehyde in 0.12 M phosphate buffer (pH 7.3, 25 °C). Following the perfusions animals were placed in the refrigerator overnight and the brains were removed on the following day, placed in cold phosphate buffer (pH 7.4) and processed for electron microscopy as detailed below.

Eight additional GEPRs were used for GAD and GABA immunocytochemistry. Briefly, 6 animals received bilateral intracerebroventricular injections of 10 μl of a 1% colchicine solution 24 h prior to sacrifice to enhance the staining of somata[30]. Three of the pretreated animals were perfused according to the protocol of Zahm et al.[39] (fixative no. 2): (1) 50 ml/rat of phosphate-buffered saline (PBS) containing 2.5% sucrose and 0.5% procaine, (2) 300 ml/rat of a fixative containing 4% paraformaldehyde, 0.1% glutaraldehyde, 2.5% sucrose in 0.07 M phosphate buffer (pH 7.4, 25 °C), (3) 100 ml/rat of PBS and 2.5% sucrose. The other 3 pretreated animals were perfused according to Mugnaini's protocol 'E'[13] (fixative no. 3): (1) 100 ml/rat of Ringer's solution (described above), (2) 300 ml/rat of 4% paraformaldehyde and 0.2% glutaraldehyde in 0.1 M phosphate buffer (pH 6.9, 25 °C), (3) 100 ml/rat of 0.1 M phosphate buffer (pH 7.2, 25 °C) and (4) 500 ml/rat of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2, 25 °C). The remaining 2 animals, that did not receive colchicine, were perfused by Mugnaini's protocol 'D'[13] (fixative no. 4): these fixative solutions have been previously used by us to study the ultrastructure of GABAergic neurons in the inferior colliculus of the Sprague–Dawley rat[33]. The interpretation of the results in the present study on the ultrastructure of the GABAergic neurons in the GEPR are based in part on results obtained from this previous study.

Immunocytochemistry

The brains to be used for immunocytochemistry were removed immediately following the perfusion, stored in cold buffer and were sectioned in the coronal plane the following day with an Oxford vibratome at a thickness of 40 μm. Sections that contained the inferior colliculus from the 8 GEPRs perfused with fixatives 2–4 were incubated in either sheep anti-GAD serum[15] or rabbit anti-GABA serum (Immunonuclear Co.) followed by subsequent incubations in reagents obtained from the appropriate sheep or rabbit avidin–biotin peroxidase kits using the recommended dilutions (ABC Kit, Vector Laboratories, Burlingame, CA). Omission of the primary antibody served as the immunocytochemical control and abolished all specific staining. Briefly, free floating sections were incubated in 0.1 M D,L-lysine in either 10% normal rabbit serum (for GAD) or 10% normal goat serum (for GABA) in 0.1 M phosphate-buffered saline, pH 7.4 (PBS) for 2–4 h followed by incubation in the primary antiserum for 24–48 h. Both GAD and GABA antisera were diluted to 1:2000 in PBS. Following this the sections were washed (three 10 min rinses in PBS) and incubated for 45 min in biotinylated secondary antibody. After another wash the sections were incubated for 45 min in the avidin–biotin peroxidase complex. Following a wash the sections were reincubated in the biotinylated secondary antibody for 30 min and washed. Next the sections were reincubated in the avidin–biotin complex for 30 min and washed. Then, the sections were reacted for 15–30 min in a DAB solution (6 mg diaminobenzidine/10 ml PBS + 0.002% hydrogen peroxide), washed and processed for electron microscopy as detailed below. These immunocytochemical methods used to study the GEPRs are identical to the methods used in our parallel study on the ultrastructure of GABAergic neurons in the inferior colliculus of the Sprague–Dawley rat[33].

Specimen preparation

Blocks were cut from the ventral lateral portion of the central nucleus from the middle of the rostrocaudal extent of the inferior colliculus from untreated sections and sections processed for immunocytochemistry. This region was chosen for analysis because this part of the inferior colliculus exhibited the most dramatic increase in cell number in GEPRs. This is the region that was analyzed in

our previous study on the ultrastructure of the GABAergic neurons in the Sprague–Dawley rat. The tissue blocks were postfixed in 1.0% osmium tetroxide for 30–60 min, dehydrated in ethanol and embedded in Epon 812. Semi-thin 2 μm sections were cut on a Sorvall ultramicrotome and stained with 0.05% toluidine blue. Serial thin sections were cut in the silver interference color range, mounted on formvar-coated slot grids, stained with lead citrate and uranyl acetate and examined with the electron microscope.

Light microscopic analysis

Random 2 μm thick semi-thin sections stained with toluidine blue were analyzed to determine the range of diameters of the small cells in the GEPR as compared to the Sprague–Dawley rat. Briefly, neurons from a region 55,225 μm^2 in size were drawn with the aid of a camera lucida from 60 samples from 3 Sprague–Dawley animals and from 53 samples from 3 GEPRs. All 6 animals were perfused with fixative no. 2, as detailed above. The diameters of the neurons were determined and the number of neurons was tabulated in 5 size groups: <10 μm, 11–15 μm, 16–20 μm, 21–25 μm and >25 μm. For this analysis 1254 and 1714 neurons were counted and classified in the Sprague–Dawley rat and GEPR, respectively. Since the increase in the density of neurons in the GEPR as compared to the Sprague–Dawley rat was obvious, it was impossible to count these specimens blindly.

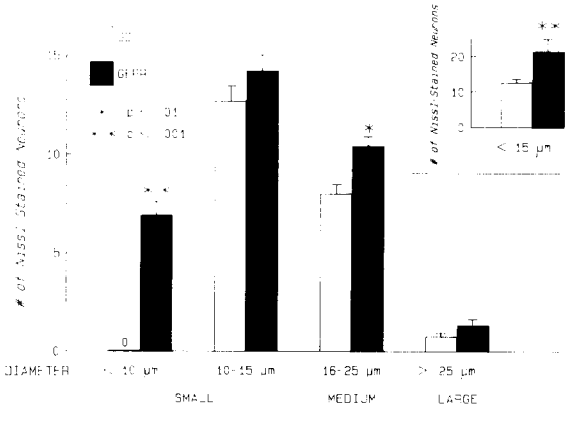

Fig. 1. Histogram of the numbers of Nissl-stained neurons in various size categories counted from 2 μm sections from the ventral lateral portion of the central nucleus of the inferior colliculus of Sprague–Dawley rats and GEPRs. Three animals were analyzed for each strain. Data were statistically analyzed using a Student's *t* test.

Electron microscopic analysis

The number of symmetric and asymmetric axosomatic and axodendritic synapses was counted for each of the 6 cell types that were described previously[27]. This analysis was based on 25 small cells, 20 medium-sized cells and 4 large cells in each of 4 Sprague–Dawley rats and 5 GEPRs. The descriptions of the ultrastructure of the inferior colliculus of the Sprague–Dawley rat are presented elsewhere[27]. The immunocytochemical preparations were examined by visual inspection to determine whether the number of axosomatic and axodendritic synapses were within the range calculated for the quantitative analysis. Where possible, the morphology of synapses in immunocytochemical preparations was classified; however, an extensive quantitative analysis was not conducted in this material. In addition, the morphology of somata and neuropil in the GEPR were described.

RESULTS

Light microscopic observations

The 2 μm thick sections revealed a heterogeneous population of neurons in the central nucleus of the inferior colliculus of the GEPR. Consistent with our previous results[33], an increase in the number of neurons was present in the GEPR as compared to the Sprague–Dawley rat. In the Sprague–Dawley preparations a laminar arrangement of somata and fibrodendritic processes was detectable and neurons were homogeneously distributed throughout the neuropil. Although the laminar arrangement was observed in the GEPR, neurons were often arranged in clusters of 3–5 neurons rather than being randomly distributed throughout the inferior colliculus as in the Sprague–Dawley rat. The size of most neurons in the clusters was small. Moreover, the neurons in the GEPR appeared to be smaller than those in the Sprague–Dawley rat as determined by visual observation.

The initial quantitative analysis used 3 cell size categories. The data (mean per unit area ± standard error) from this analysis indicated 12.7 ±

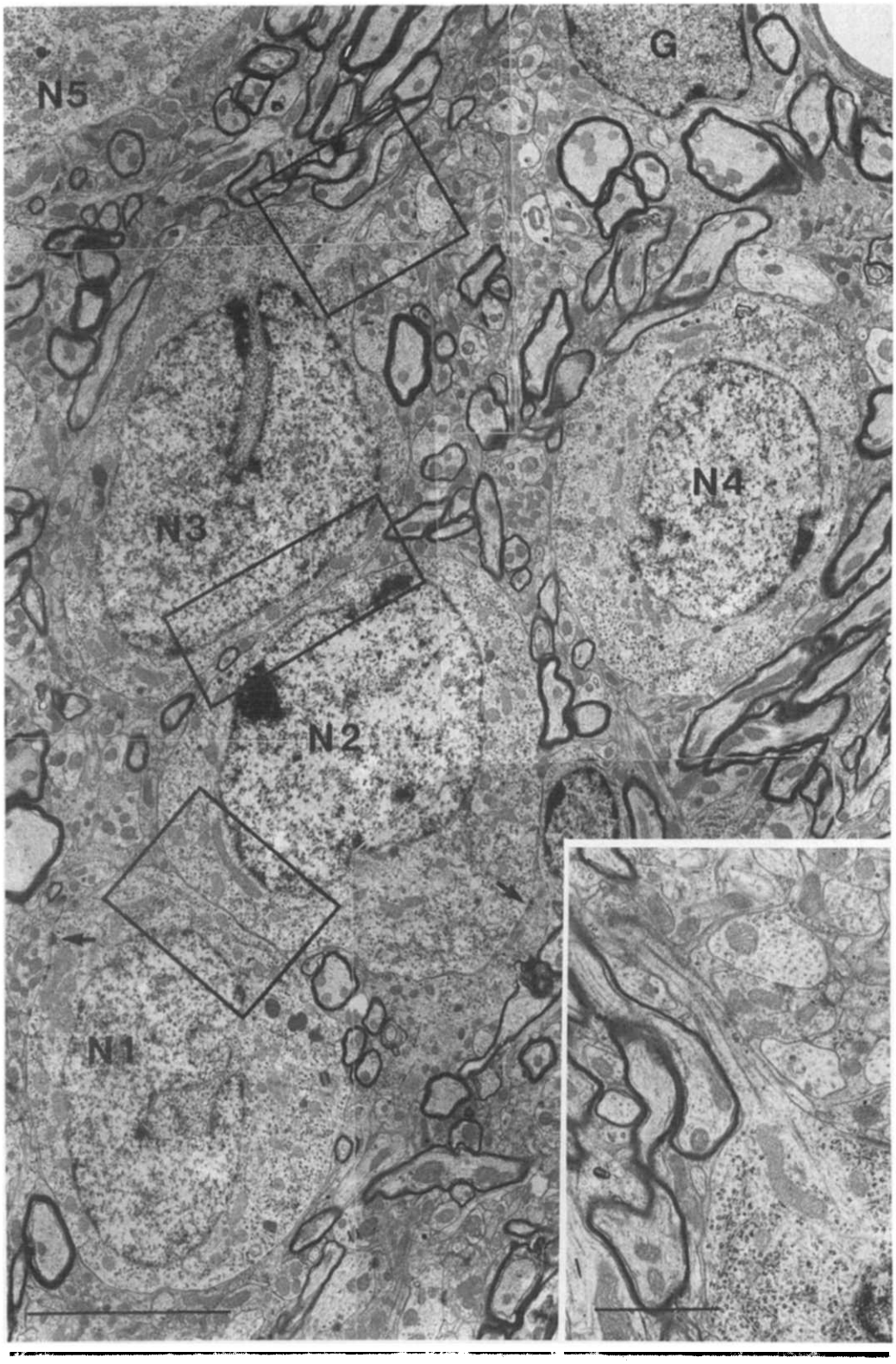

Fig. 2. A low magnification electron micrograph of the GEPR inferior colliculus showing a typical cluster formation. This rat was perfused with fixative no. 1. Neurons N1 and N2 are separated by a thin glial slip, whereas neurons N2 and N3 are separated by a thin margin of neuropil. The boxed regions are shown at higher magnification in Fig. 3. An axon initial segment emerges from N3 and the boxed area of this region is shown at higher magnification in the inset. Arrows represent synapses. G indicates glia and N5 indicates a portion of the soma of a large neuron in the adjacent neuropil. Scale bar = 5.0 μm; scale bar for inset = 1.0 μm.

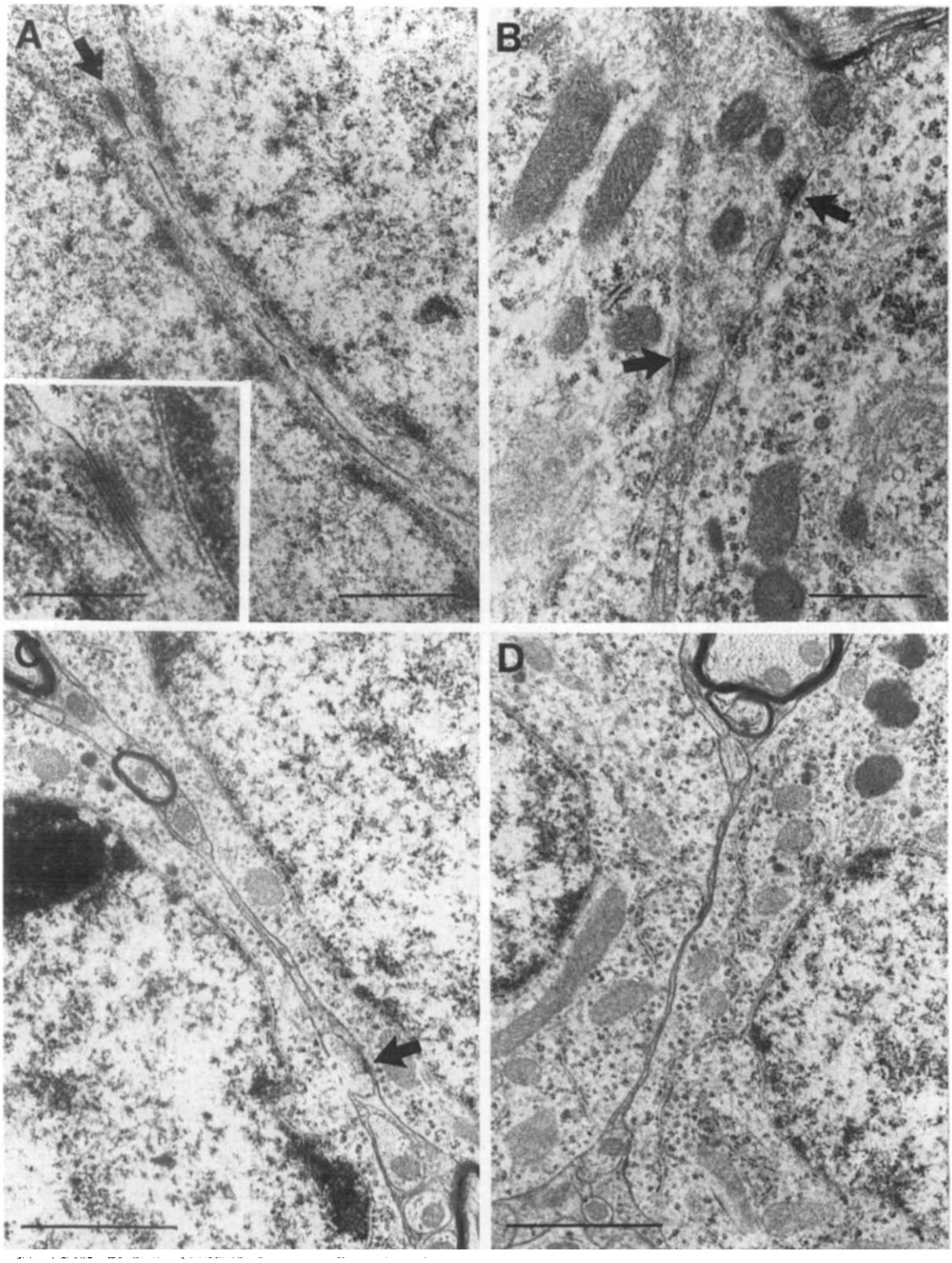

Fig. 3. Enlargements of some of the contacts between neurons in clusters (fixative no. 1). A: somata of 2 small neurons are apposed without intervening glial profiles. A contact resembling a desmosomal contact is present (arrow). Scale bar = 1 μm. This contact is shown at higher magnification in the inset. Scale bar = 0.5 μm. B: a terminal apposes the cell membranes of 2 adjacent clustered cells and forms symmetric synapses with both cells (arrows). Scale bar = 1 μm. C: an enlargement of the boxed region between neurons N2 and N3 (cf., Fig. 2). The somata of the adjacent neurons are separated by some components of neuropil, including a myelinated axon and an axon terminal which forms a synapse with one of the cells (arrow). Scale bar = 2 μm. D: an enlargement of the boxed region between neurons N1 and N2 (cf., Fig. 2). A thin glial slip intervenes between the cell membranes. Scale bar = 2 μm.

0.75 small (diameter <15 μm), 8.0 ± 0.46 medium-sized (diameter 15–25 μm), and 0.76 ± 0.16 large (diameter >25 μm) neurons in the Sprague–Dawley preparations. In contrast, the GEPR preparations displayed 21.1 ± 3.5 small, 10.4 ± 0.45 medium-sized and 1.3 ± 0.37 large neurons. The increased number of small and medium-sized neurons in the GEPRs as compared to the Sprague–Dawley rats was statistically significant ($P < 0.001$, $P < 0.01$, respectively, Student's t test).

To determine whether the neurons in the small size category in the GEPR were smaller than those in the Sprague–Dawley rat, the small cell category was divided into 2 size ranges; the medium-sized cell category was subdivided as well. In the Sprague–Dawley preparations, all small neurons were between 10 and 15 μm in diameter (12.7 ± 0.75), and there were 6.2 ± 0.46 neurons in the 16–20 μm diameter range and 2.3 ± 0.22 neurons in the 21–25 μm diameter range. In contrast, the GEPR sections displayed 6.9 ± 1.6 neurons that were 10 μm or less in diameter, 14.2 ± 0.8 neurons in the 11–15 μm diameter range, 7.4 ± 0.46 neurons in the 16–20 μm diameter range, and 3.0 ± 0.27 neurons in the 21–25 μm diameter range. Therefore, approximately 7 out of 21 small neurons, or 33% of the small neurons in the GEPR were smaller than those in the Sprague–Dawley rat. The extra medium-sized neurons in the GEPR were distributed evenly among the 2 cell size categories (16–20 μm and 21–25 μm). For a summary see Fig. 1.

Electron microscopic observations

One of the most striking features of the electron microscopic preparations of the central nucleus of the inferior colliculus of the GEPR was the clustering of neuronal somata. Clusters were formed by 3–5 neurons (Fig. 2). Neurons in clusters apposed each other in several ways as shown by the enlargements of contacts between cells in clusters (Fig. 3A–D). The cell membranes of adjacent neurons were often apposed to one another without intervening neuropil or glial slips, and contacts between cells that resembled desmosomal junctions were frequently observed (Fig. 3A). In addition, some cells were apposed to one another with only a glial slip intervening between the cell membranes (Fig. 3D). Axon terminals that were nestled between clustered neurons formed symmetric synapses with one (Fig. 3C) or both somata (Fig. 3B). Alternatively, neurons in a cluster were frequently separated by 1–2 μm of neuropil (Fig. 2). In addition to the clusters, pairs of neurons were often closely apposed to one another, usually with the cell membranes directly apposed to one another as in Fig. 3A. This juxtaposition of neurons was often formed by a large or medium-sized neuron with a small neuron. Although this latter arrangement of neurons was occasionally observed in the Sprague–Dawley rat, the frequency of this type of neuronal apposition was far more abundant in the GEPR.

Neurons with somata smaller than 10 μm in diameter had round or fusiform somata, a large nucleus to cytoplasm ratio, few stacked cisternae of granular endoplasmic reticulum, but an abundance of free polyribosomes. Approximately half of these cells had infolded nuclei (Fig. 2, neurons N1 and N3), whereas the remainder of the neurons

TABLE I

Data were analyzed with a Student's t test and no statistically significant difference was found between the number of symmetric synapses between the Sprague–Dawley (SD) or the GEPR for each cell category; the same was true for the asymmetric synapses.

Neuron size and shape of somata	*No. of asymmetric synapses*		*No. of symmetric synapses*	
	SD	*GEPR*	*SD*	*GEPR*
Very small	–	0.34 ± 0.63	–	1.92 ± 1.24
Small round	0.59 ± 1.0	0.53 ± 0.6	3.3 ± 1.9	3.1 ± 1.6
Small fusiform	0.56 ± 0.8	0.41 ± 0.17	2.8 ± 1.6	3.5 ± 2.0
Medium round	2.6 ± 3.4	0.93 ± 1.2	8.4 ± 6.2	9.0 ± 3.0
Medium fusiform	2.0 ± 3.5	2.5 ± 2.7	6.3 ± 4.1	7.3 ± 3.9
Large	5.1 ± 3.4	3.5 ± 2.0	23.2 ± 9.1	22.8 ± 5.1

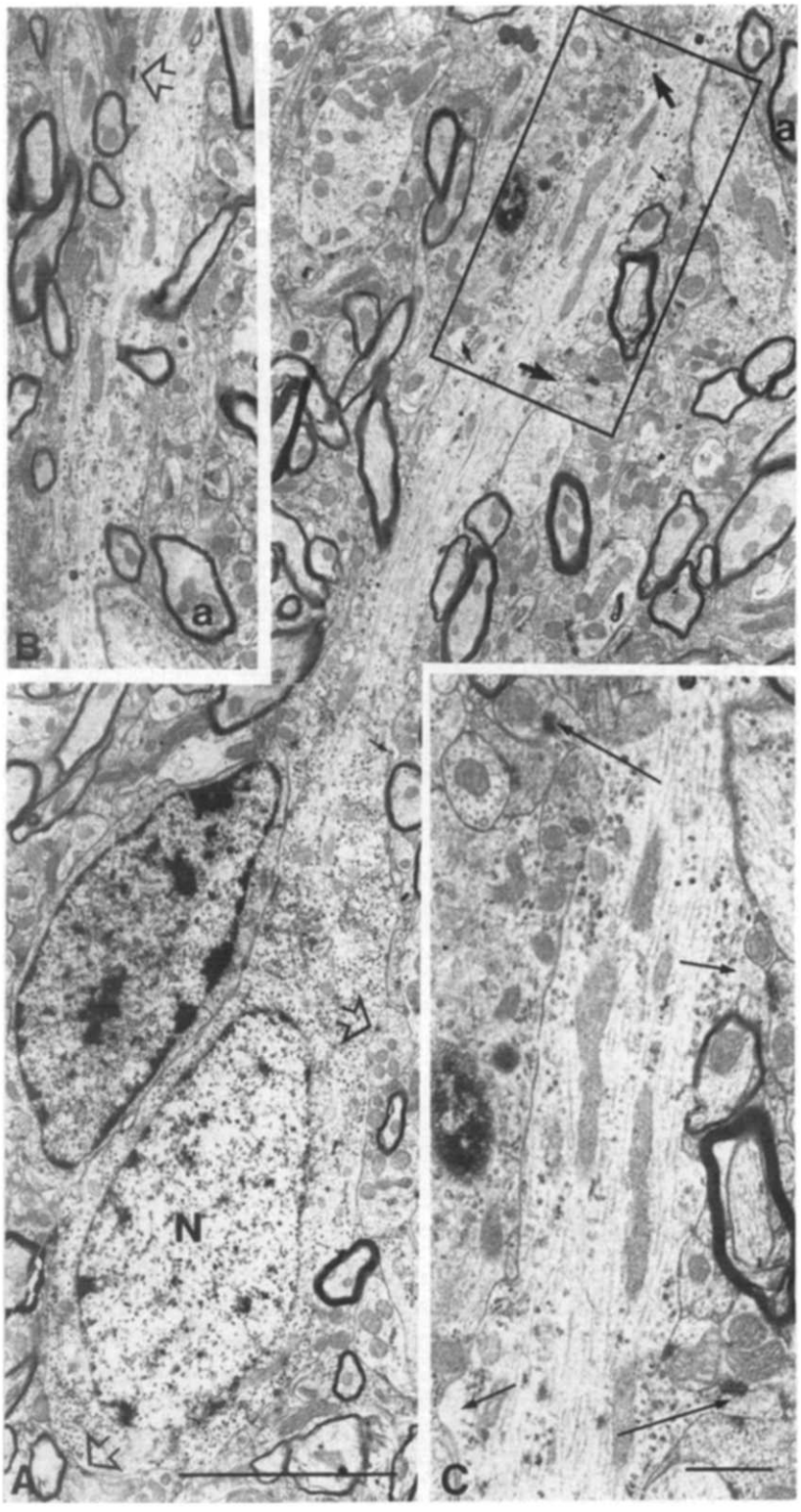

Fig. 4. A: small fusiform neuron with approximately a 35 μm segment of proximal dendrite (fixative no. 1). The myelinated axon (a) in the upper right corner of A is the same structure in the lower right corner of B. B is the distal portion of the dendrite of the neuron in A. The soma is contacted by 2 terminals (open arrows). The proximal portion of the dendrite (A and B) is contacted by only a few synapses on its shaft (open arrows). Spines approximately 1 μm in length (big arrows) as well as stubby spines (small arrows) are present on the dendrite (A). An enlargement of the boxed region is shown in C. Scale bar = 5 μm. C: an enlargement of the boxed region from A. Long spines are contacted by terminals that form asymmetric synapses (long arrows). Stubby spines are indicated by short arrows, and the one on the right side of the dendrite forms a synapse with an axon terminal. Scale bar = 1 μm.

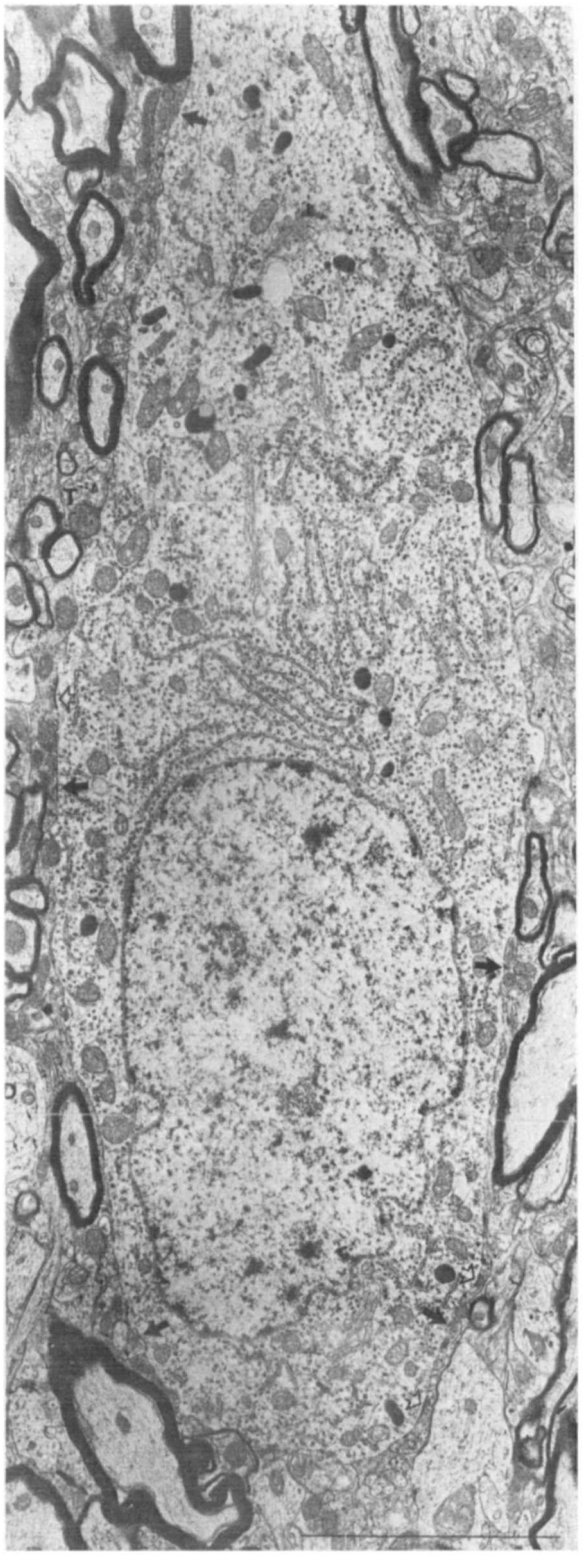

Fig. 5. Electron micrograph of a medium-sized fusiform neuron (fixative no. 1). It contains a notched nuclear envelope and the usual complement of cellular organelles with the exception of an abundance of free polysomes. Note that there are many free polyribosomes in the cytoplasm as well as stacked granular endoplasmic reticulum. Symmetric and asymmetric axosomatic synapses are indicated (solid arrows, open arrows, respectively). Scale bar = 5 μm.

had nuclei that were either notched (Fig. 2, neurons N2 and N4) or smooth (Fig. 4). The axon initial segments (Fig. 2, neuron N3 and inset) were similar in appearance to initial segments from small cells in Sprague–Dawley preparations[27], in that they were contacted by few terminals. Neuronal somata in the remaining categories had similar features as those in the corresponding categories in the Sprague–Dawley rat[27,33], except that certain cell types in the GEPR displayed more free polyribosomes and/or rough endoplasmic reticulum. These cell types included both types of medium-sized neuron (Fig. 5) and the large neurons with round somata.

The number of symmetric and asymmetric axosomatic synapses was counted for neurons in each cell category from both Sprague–Dawley and GEPR preparations. These quantitative data on the number of axosomatic synapses are reported as mean ± standard deviation of synapses in a given section rather than for the entire cell body (Table I). The average number of symmetric and asymmetric synapses on each of the cell types was similar between the Sprague–Dawley and the GEPR preparations. Proximal dendrites, followed up to 45 μm from the soma, and axon initial segments, followed up to 20 μm from the cell body, from all types of neuron were examined qualitatively for synaptic patterns. The pattern of synaptic contacts on these postsynaptic sites was similar in both the GEPR and Sprague–Dawley preparations. The dendritic shafts of small neurons received few synapses, especially those dendrites that were spiny. Spiny dendrites of small neurons (Fig. 4A and B) received asymmetric synapses on their spines and occasionally on their dendritic shafts. Proximal dendritic segments of small neurons that were not spiny received more synapses on the dendritic shaft than the spiny dendrites, and these were mainly asymmetric (not shown). In contrast to the proximal dendrites of small neurons, the proximal dendrites of medium-sized and

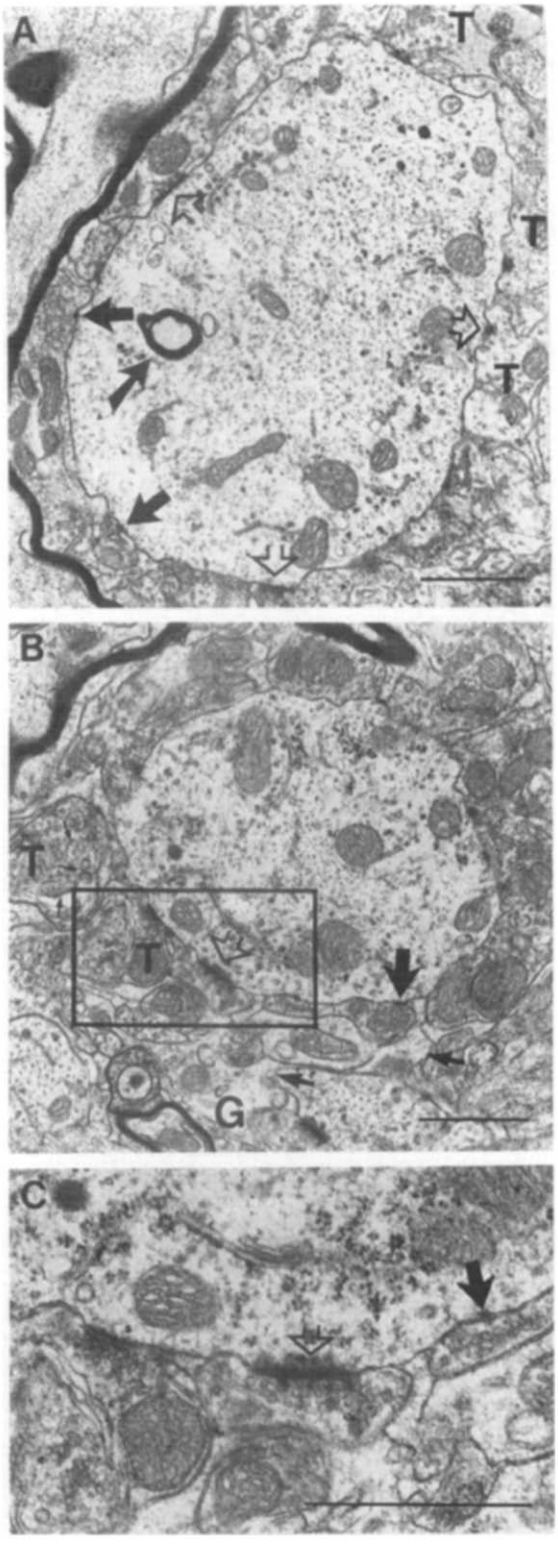

Fig. 6. A: cross-section of a normal appearing large dendrite which is contacted by several terminals (fixative no. 1). Terminals forming both symmetric (arrows) and asymmetric (open arrows) synapses are indicated (the morphology of the synapses was identified at higher magnification). Three terminals (T) that contact this dendrite are almost completely depleted of vesicles. The terminal (T) that forms an asymmetric synapse (open arrow) has a few vesicles clustered at the presynaptic density, but only a few are encountered elsewhere in this terminal. A whorl body (curved arrow) is present in the dendrite. Scale bar = 1 μm. B: a cross-section of another dendrite which displays normal morphology and is surrounded by terminals. The boxed region is enlarged in C. One of the terminals (T) contains numerous infoldings of membrane (small arrows) within it. Another terminal forms a symmetric synapse (large arrow). A glial process (G) is present in the neuropil, the extent of which is partially indicated with arrows. Scale bar = 1 μm. C: an enlargement of the boxed region in B. One terminal (open arrow) contains round vesicles and forms an asymmetric synapse. An adjacent terminal (arrow) contains flattened vesicles. Scale bar = 1 μm.

large cells were almost completely surrounded by terminals and lacked spines. The ratio of asymmetric to symmetric synapses was approximately 4:1. Thus, most of the terminals on proximal dendritic segments formed asymmetric synapses in both the GEPR (Fig. 11B) and the Sprague–Dawley rat (not shown). Axon initial segments from all cell types typically received few, if any, synaptic contacts (Fig. 2, inset). This observation was consistent with the results obtained from the Sprague–Dawley rat[27].

Pathological changes in the GEPR

A variety of ultrastructural features were observed in the central nucleus of the inferior colliculus of the GEPR that were not present or were rarely observed in the Sprague–Dawley rat. Hypertrophy of glial processes was evident in GEPR preparations (Figs. 6B, 7A, 8A and B), yet the number of glia appeared normal. As much as 1/3 of the surface area of some of the small neurons was apposed by glia in a given section (Fig. 8). Although glial profiles were present in Sprague–Dawley preparations, they did not appear to be swollen and enlarged.

A small proportion of dendrites in all size ranges exhibited marked changes in morphology in that they appeared swollen and had a watery cytoplasm (Fig. 7B). Moreover, they appeared practically devoid of microtubules, neurofilaments and had only a few mitochondria. The few neurofilaments

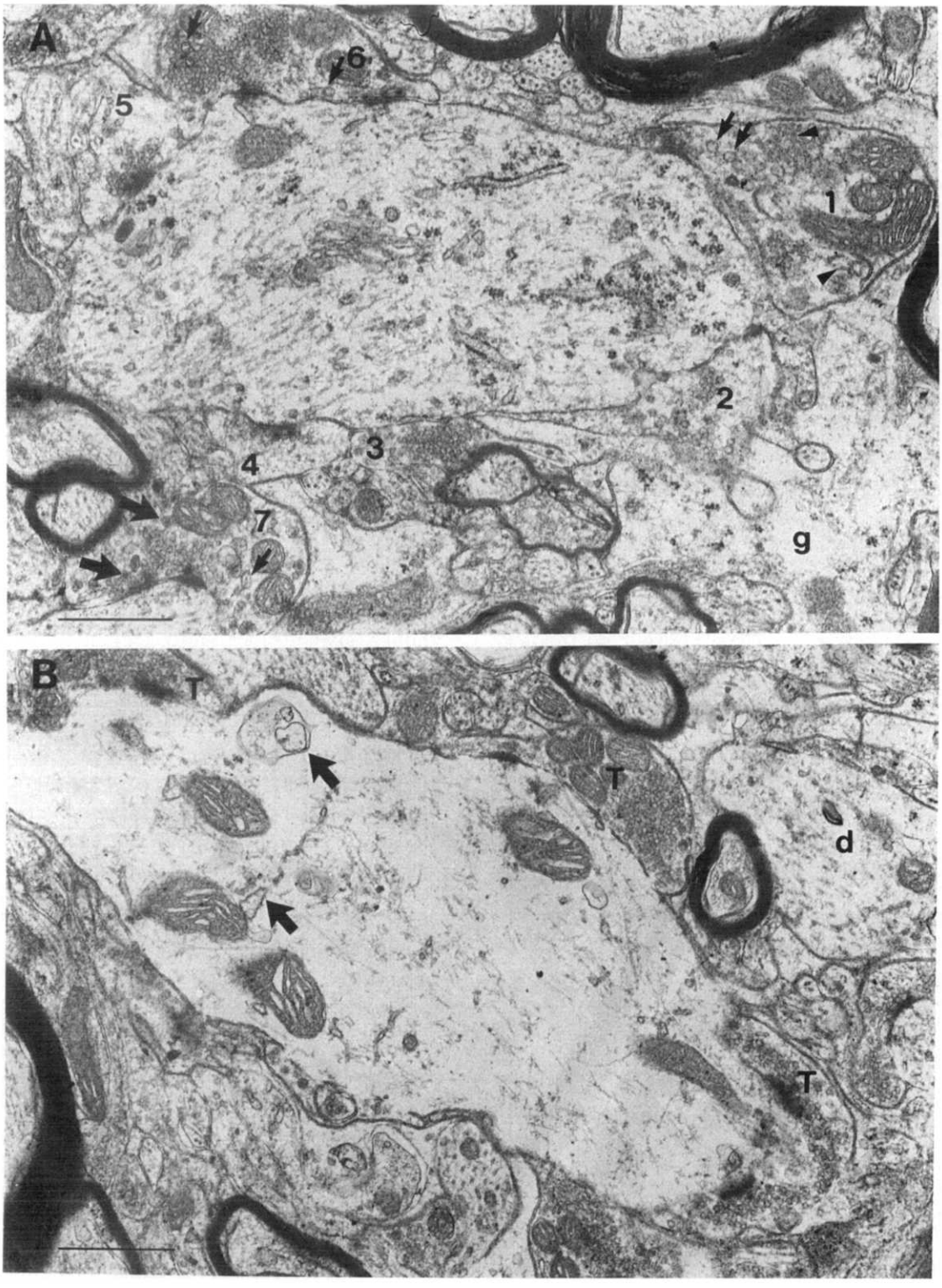

Fig. 7. A: cross-sectioned dendrite contacted by 6 terminals (1–6, fixative no. 1). The dendrite has the normal complement of organelles (compare to B). Terminal 1 contains swollen vesicles (arrows) and flattened vesicles (arrowheads). Terminal 2 is partially depleted of vesicles. Terminal 3 appears relatively normal. Terminal 4 has thickened membranes but lacks vesicles associated with the

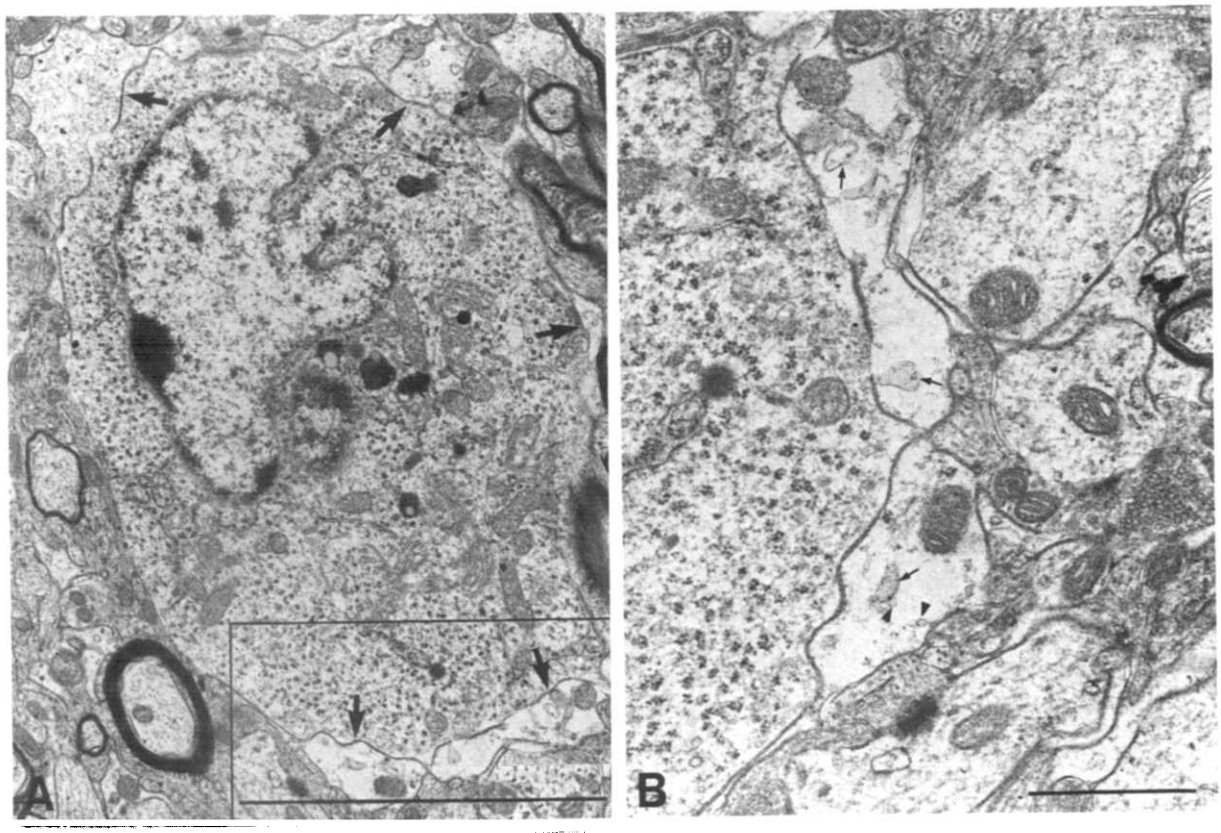

Fig. 8. A: an example of a very small neuron (fixative no. 1). Glial profiles appose the cell at several locations (arrows). Scale bar = 5 μm. B: an enlargement of the boxed region in A showing the glial profiles. Contained within the glia are mitochondria, membranes (arrows), and vesicles (arrowheads). Scale bar = 1 μm.

that were present were disorganized, rather than being arranged in parallel arrays. Such dendrites received the normal number of axodendritic synapses as determined from visual inspection; most axodendritic synapses were asymmetric. Multivesicular bodies, extra membranes and whorl bodies were very conspicuous in dendrites, axons and somata in the GEPR preparations (Figs. 6A and 7A). These inclusions and dendritic aberrations were rarely found in Sprague–Dawley preparations. Finally, terminals with swollen vesicles or terminals that were depleted of vesicles were commonly observed in the GEPR (Fig. 7A and B).

Immunocytochemical preparations

GAD and GABA immunocytochemical preparations treated with colchicine (fixed either of two ways) or not treated with colchicine were analyzed. The difference between colchicine and non-treated preparations was that the colchicine treatment enhanced the staining of neuronal somata and diminished the staining of terminals. Ultrastructural preservation was better in the tissue from animals fixed with fixative no. 3 as compared to fixative no. 2. Analysis of all tissue revealed similar types of GABAergic neurons in the GEPR as that found in Sprague–Dawley rats[33]. Thus,

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synaptic density. Terminal 5 forms a synapse but the rest of the terminal is depleted of vesicles. Terminal 6 contains swollen vesicles (arrows) as well as round vesicles. Terminal 7 contains swollen vesicles (small arrows), dense core vesicles (arrows) and small round vesicles that are associated with an asymmetric synapse. A glial profile (g) is present in the neuropil. Scale bar = 1 μm. B: profile of an aberrant dendrite contacted by several terminals. The cytoplasm is watery in appearance and lacks the regular complement of organelles (for comparison see normal dendrite (d) and cf., Figs. 6A, B and 7A). In addition membranous inclusions are present within the dendrite (arrows). Scale bar = 1 μm.

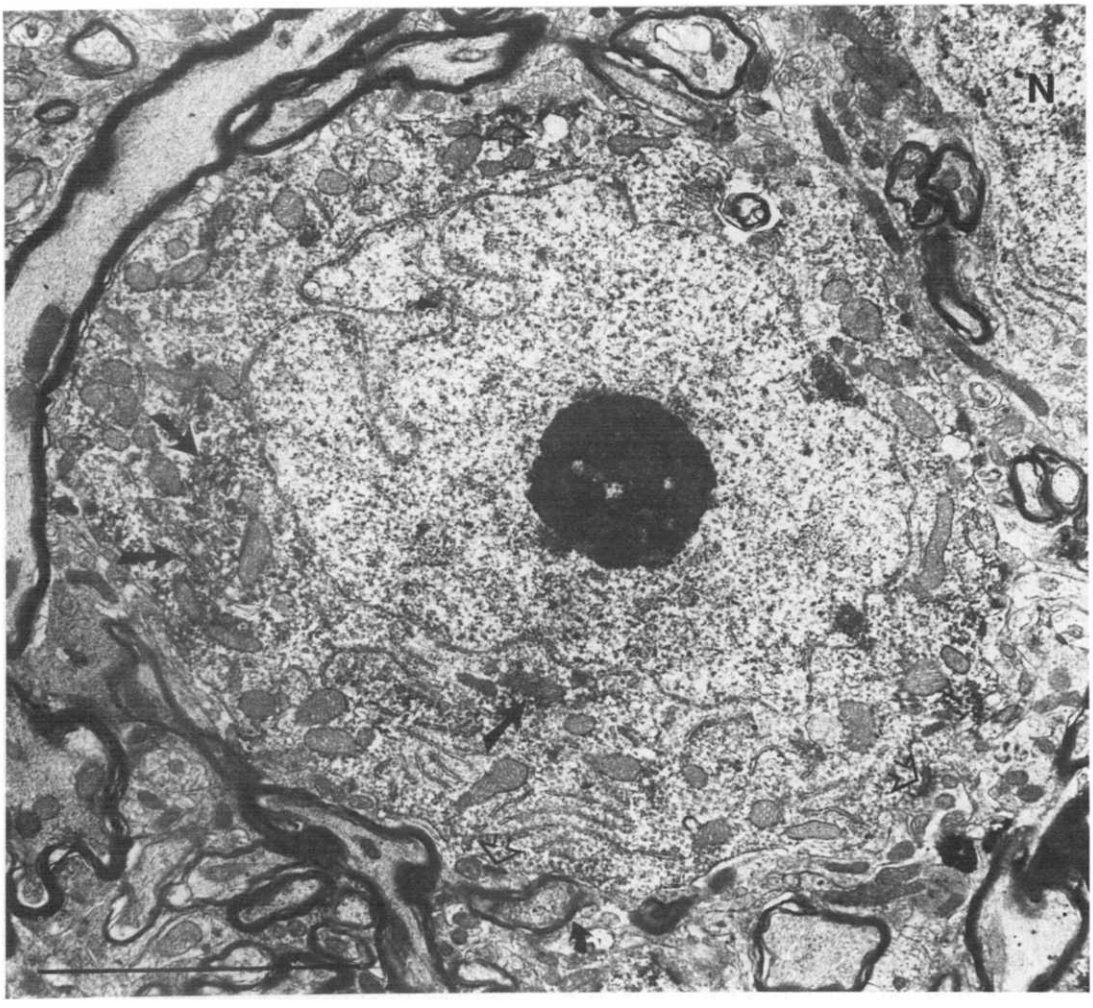

Fig. 9. A typical small GAD-positive neuron from a colchicine-treated animal (fixative no. 3). The features of this neuron include a large nucleus to cytoplasm ratio, infolded nuclear membrane and prominent nucleolus. Slanted arrows in the cytoplasm indicate reaction product. Three synapses occur on the cell body (open arrows). An adjacent soma is present nearby (N). Scale bar = 5 μm.

small, medium-sized and large multipolar cells and a small population of medium-sized bipolar neurons were immunoreactive for GAD and GABA (Figs. 9, 10 and 11). Although many of the abnormally small neurons that were described above were GABAergic, other features of the GABAergic somata, axons and dendrites in GEPRs appeared quite similar to those found in the Sprague–Dawley rats. Neurons from each size category exhibited a number of axosomatic synapses that was within the normal range found for neurons in Sprague–Dawley rats.

Synapses were often difficult to classify in these preparations because immunoreaction product often obscured the postsynaptic density. For this reason, synapses were not classified in an extensive quantitative analysis. Nevertheless, the morphology of some synapses was often identified. Our qualitative observations suggested that the somata, proximal dendrites and axon initial segments of each GABAergic cell type received similar numbers of symmetric and asymmetric synapses as did the corresponding postsynaptic targets in Sprague–Dawley material[33]. These observations are consistent with the quantitative ultrastructural analysis obtained from the routine electron microscopic preparations (Table I).

Immunoreactive elements in the neuropil from

GEPRs were similar to those in electron microscopic preparations of GABAergic neurons from Sprague–Dawley rats[33]. For example, myelinated axons were observed throughout the neuropil, and terminals that arose from these axons formed symmetric synapses (Fig. 12C). Immunoreactive terminals were found throughout the preparations and formed symmetric synapses with somata and dendrites of all sizes (Fig. 12A and B). Axon initial segments of GABAergic neurons had similar features to those in the Sprague–Dawley rat in that they received a paucity of synaptic contacts (not shown). Examples of immunoreactive aberrant dendrites (see description above) were prevalent. Aside from this latter feature, and the occurrence of abnormally small GABAergic neurons, the ultrastructural features of the GABAergic neuronal population in the GEPR appeared similar to those in the Sprague–Dawley rat.

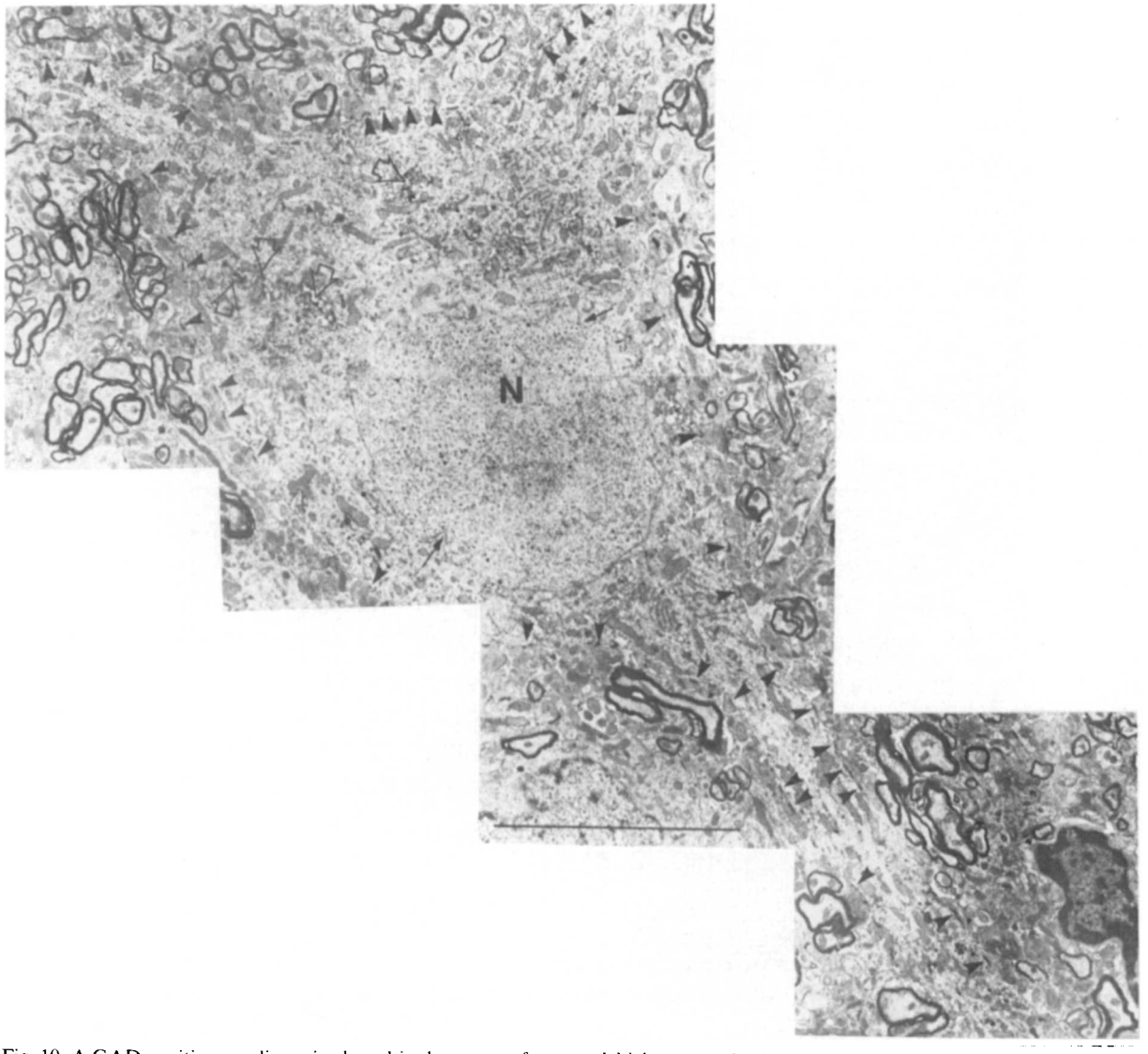

Fig. 10. A GAD-positive, medium-sized, multipolar neuron from a colchicine-treated animal (fixative no. 3). The nucleus (N) has several infoldings; reaction product is indicated by arrows in the cytoplasm. Synapses are present on the soma and proximal dendrites (arrowheads). This particular soma displays an above average number of axosomatic contacts (15) for this size neuron, however, it is still within the normal range of axosomatic contacts observed in both GEPRs and SD. Scale bar = 10 μm.

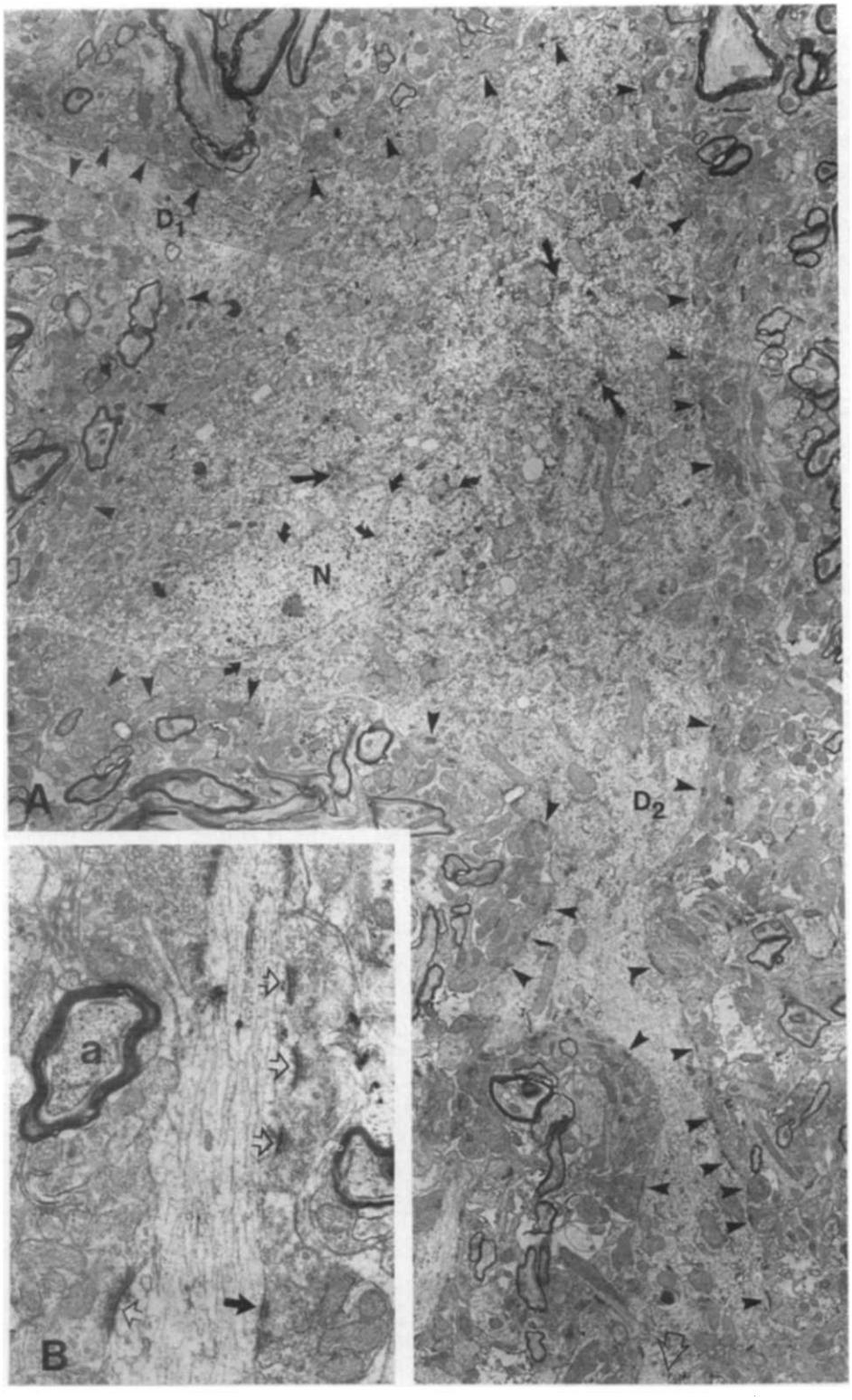

Fig. 11. A: GABA-positive large neuron from an untreated animal (fixative no. 4). The nucleus (N) is eccentrically located and displays several infoldings of the nuclear membrane (curved arrows). Immunoreactivity is indicated by slanted arrrows. The soma and proximal dendrites (D1 and D2) are contacted by many terminals (arrowheads), a finding which is normal for this size neuron. The distal portion of D2 (open arrow) is shown at higher magnification in B. Scale bar = 1 μm. B: an enlargement of the distal portion of D2 from A. Several terminals contact the dendrite and form either asymmetric (open arrows) or symmetric synapses (closed arrows). The morphology of the synapses was determined at higher magnification. An immunoreactive myelinated axon (a) is present in the adjacent cytoplasm. ×13,000.

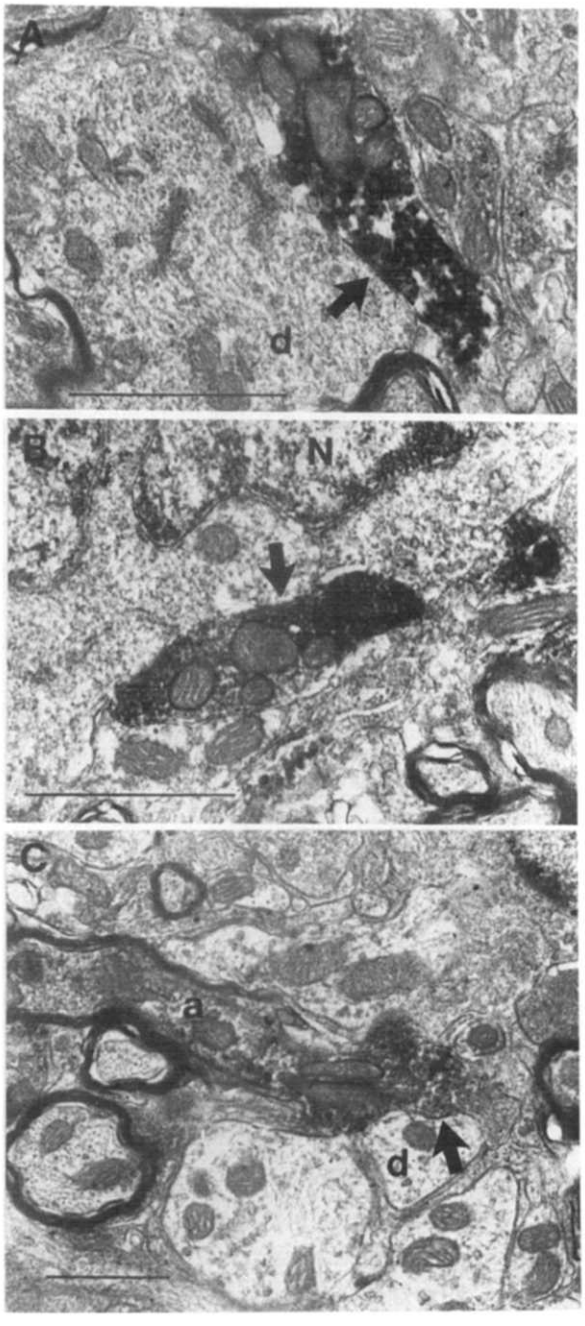

Fig. 12. GAD-positive terminals from untreated animals (fixative no. 4) A: an example of a GAD-positive terminal forming a symmetric synapse with a dendrite (arrow). Scale bar = 1 μm. B: an example of a GAD-positive terminal forming a symmetric synapse (arrow) with the soma of a small neuron. Scale bar = 1 μm. C: a terminal, arising from a GAD-positive myelinated axon, forms a symmetric synapse (arrow) with a small dendrite. Scale bar = 1 μm.

DISCUSSION

The major findings in this study were that (1) the population of small neurons in the GEPR often aggregated in clusters, (2) neurons in the GEPR received similar numbers of both symmetric and asymmetric synapses on their somata, proximal dendrites and axon initial segments as did the neurons in Sprague–Dawley rats, (3) several ultrastructural abnormalities exist in the GEPR that are not observed or are rarely observed in the Sprague–Dawley rats, and (4) the ultrastructural features of the GABAergic neurons in the inferior colliculus of the GEPR were similar to those in the Sprague–Dawley rat.

Small neurons

The results from the cell counts obtained from semi-thin sections confirmed our previous quantitative findings, in that statistically significant increases in the number of both small (70%) and medium-sized (30%) neurons occur in the inferior colliculus of GEPRs as compared to Sprague–Dawley rats[34]. The observation that many (1/3) of the small neurons in the GEPR were smaller than those found in the Sprague–Dawley rat suggests that they may be abnormal or underdeveloped.

The somatic contacts between cells in a cluster may reflect the fact that more neurons have been packed into the same volume of inferior colliculus as that found in Sprague–Dawley rats. It is unclear if these numerous somal appositions are functionally significant. In other brain regions where a high packing density of cells is found (i.e., granule cells in the hippocampus), membranes of adjacent cells are closely apposed[29] as they are in the inferior colliculus of the GEPR. Thus, this cluster arrangement in the inferior colliculus of GEPRs may or may not be related to audiogenic seizures.

Synapses in the IC

Another purpose of the present experiment was to determine whether there was an ultrastructural basis for a circuitry that may support the disinhibition hypothesis as discussed in the Introduction[21]. Although previous data for the seizure-sensitive gerbil showed an increase in the number of terminals that form symmetric axosomatic synapses on

basket cells as compared to seizure-resistant animals[20], the results in the present GEPR study are different.

The quantitative data for axosomatic symmetric synapses for neurons in the inferior colliculi of GEPRs did not indicate a circuitry for disinhibition because no significant differences were found between Sprague–Dawley rats and GEPRs. Moreover, a unimodal distribution of the number of axosomatic synapses occurred for each of the cell size categories. The curve of this distribution for the GEPR closely conformed to that for the Sprague–Dawley rat. In addition, our qualitative analysis did not suggest any differences in the number of symmetric synapses on proximal dendrites or axon initial segments in the inferior colliculus. Therefore, a circuitry for disinhibition is probably not present in the ventral lateral portion of the central nucleus of the inferior colliculus. It is possible that a disinhibition circuit may be present either in another subdivision of the inferior colliculus or in a brain region that receives a major projection from the inferior colliculus.

In the central nucleus of the inferior colliculus of the GEPR, the type of GABAergic neuron that is increased in number is presumed to be an interneuron. This assumption is based on descriptions of a locally arborizing axon from small multipolar[35,36] or stellate neurons[16]. However, Oliver and Morest[16] pointed out that these neurons could be projection neurons with a locally arborizing collateral system. Although these latter studies were conducted in the cat and the present study used rats, Faye-Lund and Osen[5] have stated that the morphology of the neurons in the central nucleus of the inferior colliculus of the rat resembles that found in the cat. Thus, the possibility exists that the supernumerary GABAergic neurons in the inferior colliculus of the GEPR may form abnormal connections in brain regions that are targets of the inferior colliculus. However, the relationship of the increased number of GABAergic neurons to the seizure activity remains to be determined.

It is interesting that the seizure-sensitive gerbil displays a circuitry for disinhibition but a similar circuitry was not evident in the central nucleus of the inferior colliculus of GEPRs even though both models contain increased numbers of GABAergic neurons in a specific brain region. If a disinhibition circuit is not present somewhere in the GEPR as discussed, then the GABAergic defect in the GEPR is different than that found in the seizure-sensitive gerbil. However, not all genetic models are identical in the types of transmitter defects they contain. For example, models that share the same epileptogenic stimulus, such as the GEPR and the audiogenic seizure susceptible mouse, show differences for the role of dopamine and norepinephrine in seizure modulation[7–9]. Therefore, it is not surprising that a difference in circuitry or in the role of the extra GABAergic neurons may exist between the GEPR and the gerbil.

Ultrastructural anomalies

One of the differences found for the somata of neurons in the inferior colliculus of the GEPR as compared to those in the Sprague–Dawley rat is that more free polyribosomes are present in large neurons with round somata and in both shapes of medium-sized neuron in the GEPR. Also, the very small neurons found only in the GEPR contain an unusually high density of free polyribosomes. These results were consistent with light microscopic observations in that neurons in the GEPR are more intensely stained in Nissl preparations than those in the Sprague–Dawley rat[31]. This suggests that neurons in the GEPR may be synthesizing proteins more actively than those in the Sprague–Dawley rat, and it may also reflect the increased physiological activity of this structure in the GEPRs.

The pathological changes observed in the central nucleus of the inferior colliculus of the GEPR were similar to those described in certain experimental models of epilepsy, such as those induced by sustained electrical stimulation[17], lesions[22] and convulsant drugs, such as kainic acid[19,37], dipiperidinoethane[18], and folic acid[18]. The prevalence of extra membranes may signify increased membrane recycling. This finding has been described in other models of epilepsy and has been interpreted to reflect an increase in synaptic activity[17]. Hypertrophy of glia and swollen or empty dendrites has been attributed to excitotoxic effects of afferents to a brain region[17–19]. Our data indicate that the dendrites in the inferior colliculus receive a higher

concentration of asymmetric synapses than do somata or axon initial segments. This implies that dendrites are exposed to more excitation per unit area than somata or axon initial segments, and may explain why this type of morphological change is observed only in dendrites rather than in axons and somata.

For several reasons it is unlikely that the appearance of empty dendrites is due to inadequate fixation. Ultrastructural perturbations occurring as a result of poor fixation do not resemble that of the empty dendrites. Moreover, this type of dendrite is almost never observed in Sprague–Dawley material which was prepared in the same way. In addition, empty dendrites occur in fields where the morphology of adjacent profiles is excellent. Therefore, the empty dendrites in the inferior colliculus of GEPRs represent a pathological change rather than an artifact of inadequate fixation.

The occurrence of empty dendrites, swollen vesicles, multivesicular bodies, whorl bodies and extra membranes in the GEPRs is not as frequently observed as in other experimental models. However, animals from other experimental models are usually examined shortly after the seizures whereas the animals used in the present study presumably did not have seizures for a week. Pico et al.[22] made a time course analysis of ultrastructural changes that occur in the hippocampus after hilar lesions and suggested that the most dramatic changes occur immediately after seizures. In contrast, they showed that after longer time intervals between seizures and sacrifice, the ultrastructural features approach normality. Thus, it is reasonable to expect that GEPRs sacrificed closer to the time of the last seizure would exhibit similar changes as described in the present study, but an increase in prevalence of these changes would be noted.

The significance of these findings is that similar changes occur in brain regions exhibiting epileptic activity or in regions along the pathway of seizure spread and thus, these findings suggest that the inferior colliculus is related to either the propagation or amplification of seizure activity in the GEPR. Recent studies have shown that an increase in excitatory amino acid transmitters are present within[25] or are released from[1] the inferior colliculus of GEPRs as compared to Sprague–Dawley rats. These biochemical data, taken together with our observations on the anatomical anomalies that resemble excitotoxicity in the inferior colliculus of the GEPR, suggest a possible role for excitatory amino acids in audiogenic seizures in the GEPR.

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